Original Article

A Cannabinoid Type 1 Receptor Antagonist Impairs Spatial Memory and Increases the Tau Gene Expression in an Animal Model of the Alzheimer's Disease

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ABSTRACT

Alzheimer's disease is a neurodegenerative disease that is characterized by the accumulation of two different proteins, β-amyloid and tau. The objective of the present study was to examine the impact of bilateral administration of the cannabinoid receptor antagonist (AM251) in the hippocampus on spatial memory and tau gene expression in an Alzheimer's disease model. The β-amyloid toxin was administered bilaterally into the hippocampus of Wistar male rats to induce Alzheimer's disease. The rats were then divided into four groups: the control group (which received distilled water as a solvent for β-amyloid toxin), the lesion group (which received the β-amyloid), β-amyloid + DMSO group (as antagonist solvent), and the AM251 antagonist receiving groups. During the training course of the Morris water maze test, the antagonist of the cannabinoid 1 receptor antagonist AM251 was administered bilaterally into the hippocampus for four consecutive days at doses of 5, 25, and 100 ng. To evaluate the spatial memory of the animals, the following parameters were analyzed: distance traveled, latency time to reach the hidden platform, velocity of the animals, and tau gene expression in real time. The spatial memory indices were found to be impaired following the injection of β-amyloid and the AM251 cannabinoid antagonist. Following the injection of β-amyloid toxin, there was an increase in mRNA expression of tau protein. However, no significant difference was observed between the cannabinoid antagonist and β-amyloid groups. These results indicate that β-amyloid toxin has a destructive effect on spatial memory and that cannabinoid system plays a positive role in memory formation and consolidation, However, further studies are needed to confirm these findings.

Keywords: Alzheimer's disease, Cannabinoid Antagonist, Spatial Memory, Tau Protein

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1. Introduction

Alzheimer's disease is a neurodegenerative disorder and the most prevalent cause of senile dementia, which continues to affect inflict millions of people worldwide. This disease is an irreversible progressive brain disorder with an unspecified etiology, accompanied by severe learning and memory difficulties. In Alzheimer's disease, the neuronal cell structure is destroyed, which affects memory and behavior. The initial symptoms of the disease manifest as a gradual impairment of memory, particularly spatial memory. This disease presents a significant challenge from both a medical and social perspectives, with an unmet need for effective therapeutic approaches. There is a growing body of research exploring potential strategies for its prevention and treatment (1). In this type of disease, abnormal proteins accumulate within and outside of the neuronal cells, impeding neuronal network communication, and ultimately leading to demise of specific neurons. Alzheimer's disease is characterized by the accumulation of two distinct proteins: one situated outside the neuron al cells and the other within them. A protein extracted from neurons is designated as amyloid-β (Aβ), while the collective within neurons is referred to as tau. The accumulations of beta-amyloids is referred to as amyloid plaques, which are highly toxic and disrupt with the function and communication of neurons. In Alzheimer's disease, there is an accumulation of tau protein within neurons, which is characterized by the formation of neurofibrillary tangles (NFTs). Neurofibrillary tangles gradually accumulate within the cell until it is entirely filled and eliminated. The formation of plaques, the release of acetylcholinesterase enzymes from the plaques, the occurrence of inflammation in the brain tissue, and the toxic effect of amyloid deposits on brain cells are the primary causes of injuries to the brain. One of the neuropathological characteristics of Alzheimer's disease and a contributing factor to neuronal degeneration, particularly in the hippocampus, is the presence of entangled neural fibers comprising tau protein in brain cells (2). Tau proteins ehance microtubule stability, which is diminished by phosphorylation. In other words, the normal state of this protein preserves the stability of the microtubules that constitutes the neural pathways, thereby preventing their disruption (3). However, following hyperphosphorylation, hyperphosphorylated protein formations are produced, resulting in mixed nerve fibers within the neurons. It should be noted that neither amyloid plaques nor neurofibrillary tangles are exclusive to Alzheimer's disease. However, they may serve as indicative markers of Alzheimer's disease (2). Additionally, the cannabinoid system has been demonstrated to influence memory and learning processes (4-7). Several studies have demonstrated the presence of cannabinoid 1 in various brain regions, including the hippocampus, thalamus, cortex, cerebellum, and limbic system. Additionally, CB2 receptors have been identified in the immune system cells and recently in the CA1 and CA3 areas of the hippocampus (8-10). In this regard, countless studies have been conducted with the objective of identifying an efficacious approach to postpone or treat Alzheimer's. Additionally, the effects of cannabinoids on acetylcholine release in the hippocampus and the presence of muscarinic and cannabinoid receptors have been established (8, 11-13). Furthermore, studies have demonstrated that the genetic deletion of cannabinoid receptors during the early stages of rat development results in memory impairment and a cognitive function decline. This rapid reduction in cannabinoid receptors is accompanied by the loss of neurons in the CA1 and CA3 regions of the hippocampus (11, 14). Moreover, the effects of cannabinoid receptor 1 antagonists on spatial memory and genes involved in Alzheimer's disease have been investigated in only a limited number of studies. In light of the pivotal role of cannabinoids in all phases of memory consolidation, the present study sought to examine the effects of bilateral administration of varying doses of AM251, a cannabinoid 1 receptor antagonist, on spatial memory and tau gene expression in an AD model.

2. Materials and Methods

2-1. Animals

The experiments were conducted on adult male Wistar rats weighing 250-200 g, sourced from the Pasteur Institute, Tehran, Iran. The animals were housed in groups of four per cage and provided with food and water ad libitum. The room was maintained on a 12-hour light/dark cycle and a controlled temperature (23 ± 2°C). The rats were randomly assigned to experimental groups of eight. The study protocol was approved by the ethical committee of Alborz University of Medical Sciences, Iran.

2-2. drugs

Aβ fragment 25-35 and AM251, an antagonist of the cannabinoid 1 receptor, were procured from Sigma. It should be noted that the cannabinoid receptor agonist drug Win55 was purchased from a commercial source,, but was subsequently denied entry into the country due to international sanctions imposed on Iran. Given the unavailability of an ideal agonist, in lieu of one dosage, three distinct doses of AM 251 were selected.

2-3. Experimental Procedure

In this study, rats (n = 40; n=8 in each group) were randomly divided into the following groups: a control group, treated with distilled water as the solvent of Aβ;a DMSO group treated with DMSO as the solvent of AM251; and a treatment group that received AM251 at doses of 5, 25, and 100 ng/ml. The treatment was administeredinto the dentate gyrus of the hippocampus for four days, accompanied by training courses for the Morris test. For the purposes of stereotaxic surgery, the rats were anesthetized via intraperitoneal injection of chloral hydrate (80 mg/kg) and subsequently placed within a stoelting stereotaxic apparatus (USA). The scalp was cleansed with iodine solution and incised along the midline, after which a burr hole was drilled through the skull. The animals in the Aβ group were bilaterally injected with a solution

containing 10 µg aggregated Aβ (25–35) (5µg/µl, Sigma, USA) in the dentate gyrus of the hippocampus (at coordinates 2.8 mm posterior to bregma, 2 mm lateral to sagittal suture, and 2.8 mm below dura) (15) to induce Alzheimer's disease. The animals in the control group received of the same volume of distilled water. To facilitate the formation of neurotoxic Aβ fibrils, Aβ was dissolved in distilled water and the resulting solution was incubated at 37°C for 3 days. Following the surgical procedure each rat was housed individually in a clean, pre-disinfected cage. In this study, a five-day protocol was employed to assess both the Morris water maze and tau gene expression.

2.4. Behavioral Testing

The Morris water maze apparatus consisted of a black, rounded basin with a diameter of 136 cm and a height of 60 cm, filled to a depth of 25 cm with a water maintained at a temperature of 20 ± 1°C. The maze is situated within a room that features various visual cues, including a clock, window, poster, shelf, and lighting. These elements are hypothesized to contribute to the perception of the maze as a four-quarter circle structure. A circular platform, constructed from o plexiglass with a diameter of 10 cm, was positioned in the center of one of the quarter-circles below the surface of the water. A diode emitting infrared light will be affixed to the animals' back via rubber tape of the animal, and a video camera— capable of detecting infrared light—will be positioned at the top of the basin. Subsequently, the signals were transmitted to the computer and analyzed using the software system. The latency time and distance traveled by the animal to find the hidden platform were measured.

2.4.1. Training of the Animals

In the experiments conducted on the hidden platform, each rat was trained for four days. On each day, one block was trained four times. In each block, the animal was released four times, with each release occurring in a randomly determined direction (north, south, east, and west). A period of 90 seconds was allotted for each rat to flocate of the platform. If the animal was unable to do so, it would be directed towards the platform.In all cases, a further 30 seconds was permitted for the animal to remain on the platform and explore its surroundings.

2.4.2. Evaluation of Health of the Sensory-Motor System

On the fifth day of the test, the platform was covered with aluminum paper and placed at an approximate height of one centimeter above the water surface until it was fully visible. The experiment, designated the "visible platform", was conducted to ascertain whether the animal was able to locate the platform, thereby confirming the health of its visual-motor system. At the end of each experiment, animals were dried and transported to their cages. The distance traveled by the animal to reach the platform and the time elapsed before reaching the platform are regarded as indices of learning and spatial memory. Furthermore, the speed at which the animal swims is an indicator of the health of its sensorimotor system.

2.5. RNA Extraction, cDNA Synthesis and Quantitative Real time-PCR (qRT-PCR) Analysis and Evaluating Tau Gene Expression

Following anesthesia, the hippocampus of the animals was removed and stored at -80°C for subsequent PCR performance. Subsequently, total RNA was extracted from the hippocampal tissueusing RNX+ extraction solution (Cinnagen Company, Iran), following the chloroform-alcohol protocol. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed using Taq polymerase and specific forward and reverse primers for the tau protein gene, as listed in table 1. Finally, the samples were subjected to electrophoresis, and gene expression levels was quantified using the QIAGEN Real-Time PCR Detection System. Normalization was conducted using the mean expression of the B2M gene, which exhibited the most stable index. The relative quantification of tau was performed using the $2^{-\Delta\Delta CT}$ method.

2.6. Statistical Analysis

The data were analyzed using SPSS version 22 software.A One-way ANOVA was employed to compare the mean values of the independent groups. The Tukey post hoc test was used for the purpose of verifying the differences between the different groups, and $p<0.05$ was considered to be statistically significant.

3. Results

In this study, an animal model of AD was established by intra-hippocampal injection of Aβ.The distance traveled and time latency to find the hidden platform, and the velocity of the animals in the Morris water maze apparatus, were measured. One-way ANOVA demonstrated that treatment with AM251 100 ng resulted in a statistically significant increase in the distance traveled to reach the hidden platform in the Morris water maze apparatus when compared to rats that received Aβ ($F(5, 42) = 13.31$, $p<0.001$). Furthermore, treatment with AM251 100 ng resulted in a significantly greater increase the distance traveled compared to the groups treated with AM251 (5 and 25 ng) (both $p < 0.01$) (Figure 1). The One-way ANOVA revealed that treatment with 100 ng of the cannabinoid antagonist AM251 led to a notable increase in latency time (the time taken to reach the hidden platform) [$F(5, 42) = 10.10$, $p<0.001$] in the Morris water maze test compared to rats receiving Aβ (Figure 2). This increase was statistically significant ($F(5, 42) = 10.10$, $p<0.001$). Tukey post hoc analysis revealed a statistically significant difference between the DDW group (the distilled water-treated group) and the Aβ group ($p < 0.05$). The latency time was found to be significantly higher in the group treated with 100 ng of cannabinoid antagonist AM251, the latency time was significantly higher than that in the Aβ-treated group ($p<0.01$). Furthermore addition, there was a significant increase in the latency time was observed between the AM251 5 ng and AM251 100 ng groups ($p<0.05$) (Figure 2).

Table 1. The stem loop primers sequences for cDNA synthesis and qRT- PCR.

Gene	Primers	Sequence	Tm (ºC)
B2M	Forward	GCTATCCAGAAAACCCCTC	60
	Reverse	CCCGTTCTTCAGCATTTG	
TAU	Forward	AAGTGTGGCTCATTAGGCAAC	60
	Reverse	ACCACTGGCGACTTGTACAC	

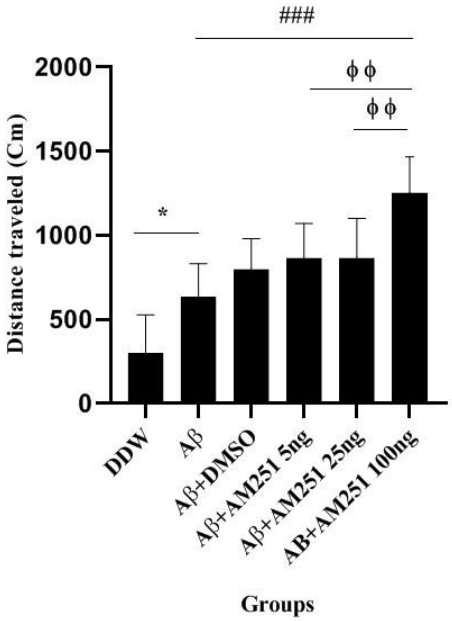

Figure 1. Distance traveled to reach the hidden platform in the Morris water maze following cannabinoid antagonist AM251 treatment. The distance traveled in the Aβ-treated group increased significantly compared with that in the DDW group. Treatment with AM251 100 ng increased significantly the distance traveled to reach the hidden platform compared to rats receiving Aβ. In addition, treatment with AM251 100 ng increased significantly the distance traveled compared to the AM251 5 and 25 ng treatment groups. Data are presented as mean ± SEM (n = 7 in each group). *: $p < 0.05$ vs DDW group; ###: $p < 0.001$ vs Aβ - received group; φφ: $p < 0.01$ vs antagonist AM251 5 and 25 treatment groups.

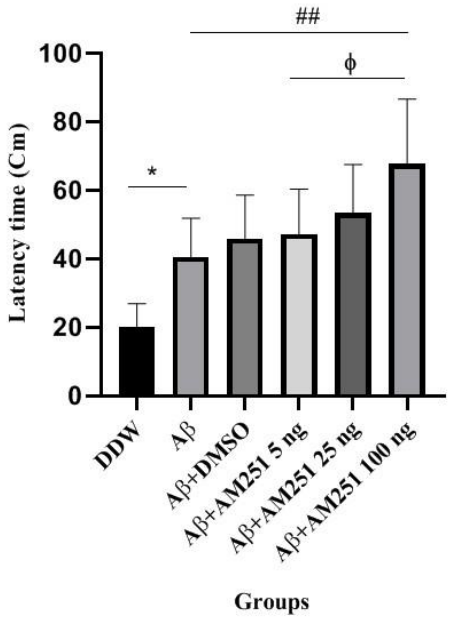

Figure 2. Latency time to reach the hidden platform in the Morris water maze following cannabinoid antagonist AM251 treatment. The latency time in the Aβ - receiving group increased significantly compared to that of the DDW group. In the group treated with 100 ng of cannabinoid antagonist AM251, the latency time was significantly higher than that in the Aβ-treated group. In addition, there was a significant increase in the latency time between the AM251 5 ng and AM251 100 ng groups. Data are presented as mean ± SEM (n = 7 in each group). *: $p < 0.05$ vs DDW group; ##: $p < 0.01$ vs Aβ - received group; φ: $p < 0.05$ vs antagonist AM251 5 treatment group.

One-way ANOVA indicated that the velocity (speed) of the animals reaching the platform did not show any significant differences among the studied groups (Figure 3). One-way ANOVA revealed that the expression of the tau gene in the groups receiving different concentrations of the cannabinoid antagonist AM251 (5, 25, and 100 ng) did not decrease significantly compared to that in the Aβ-receiving group (Figure 4).

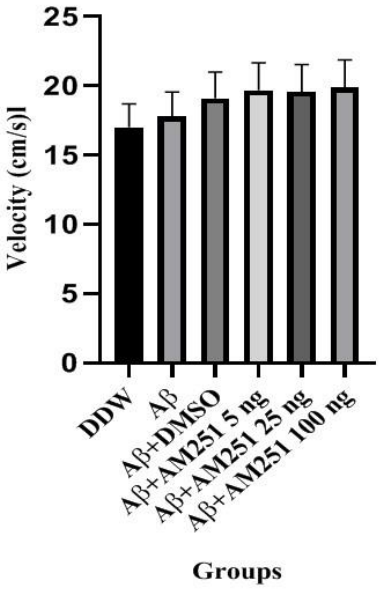

Figure 3. Velocity of animals to reach the hidden platform in the Morris water maze following cannabinoid antagonist AM251 treatment. There were no significant differences between the groups. Data are presented as mean ± SEM (n = 7 in each group).

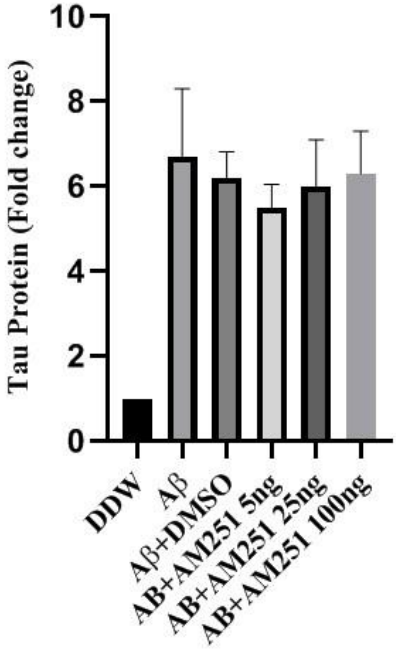

Figure 4. Expression of the tau gene in the groups receiving different concentrations of cannabinoid antagonist AM251 (5, 25, and 100 ng). There was no significant decrease between the Aβ - and the AM251- trated groups. Data are presented as mean ± SEM (n = 7 in each group).

4. Discussion

Alzheimer's disease is an active neurodegenerative disorder that is observed in individuals with advanced age-related cognitive decline. A number of factors are involved in the development of this disease including the toxicity of amyloid plaques to brain tissue and plaque inflammation, are involved in the development of this disease. The progression of Alzheimer's is associated with a decline in behavioral and cognitive function including, memory, spatial memory, and the ability to navigate (1, 16, 17). The hippocampus, in particular the dorsal part of the hippocampus (CA1), represents a significant component of the human brain, as well as that of other vertebrates, and mammals. Damage to this area result in significant memory impairments, including forgetfulness, Alzheimer's disease, and other memory-related issues and problems (6, 18, 19). A substantial body of research has demonstrated the presence of a considerable number of CB1 cannabinoid and serotonin receptors in GABAergic neurons within the amygdala and hippocampal formations (20, 21). The objective of this study was to investigatethe effects of bilateral administration of a cannabinoid antagonist (AM251) in the hippocampus on spatial memory and tau gene expression in an Alzheimer's disease model were investigated. In this study, an intra-hippocampal beta-amyloid injection of beta-amyloid (35-25) was utilized to induce Alzheimer's disease.The results of the Morris water maze test indicated that, during the acquisition and consolidation phases, the rats in the β-amyloid receiving group travelled longer distances and spent more time reaching the target platform than the control group (Figures 1&2). This finding supports the hypothesis that β-amyloid impairs learning. It has been demonstrated that the cannabinoid CB1 receptor agonists may influence the processes of acquisition and memory consolidation (22, 23). A substantial body of evidence indicates that the cannabinoid receptors may be negatively coupled with Gi/o proteins via Adenylyl cyclase, thereby reducing the production of cAMP. It has been demonstrated that CB1 typically functions through this G-protein on the to pre-synaptic-N-type-voltage related calcium channels, which signifies the role of cannabinoids in regulating the CNS neuron transmissions (24). Additionally, it has also been documented that endocannabinoids are released from post-synaptic locations and have the capacity to inhibit the release of other transmitters, including serotonin, GABA, opioids, and dopamine. This may result in significant and long-term alterations in various behavioral patterns (25, 26). Following the administration of three different doses of AM251 cannabinoid antagonists (5, 25, and 100 ng) to the animals, a notable correlation was observed between the latency time to reach the hidden platform and the administered dose (Figure 3). The findings demonstrated a notable elevation in memory impairment among the experimental subjects. In this study, the administration of various concentrations of cannabinoid receptor 1 antagonist AM251 (5, 25, and 100 ng) resulted in an elevation inTau gene expression within the experimental groups, in comparison to the Alzheimer's model group, which did not receive any drug. The precise mechanism by which cannabinoids act within the hippocampus remains unclear. However, endocannabinoids may act as mediators that modulate the regulation of diminished GABA release in the hippocampus (27, 28). In light of the findings presented in this study and previous findings (29), it can be concludedthat the administration of a cannabinoid antagonist has a significant impact on the deterioration of spatial memory and the enhancement of tau gene expression. Nevertheless, additional research is required in this field, particularly in the context of cannabinoid agonists.

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Authors' Contribution

Study concept and design: B.TF.
Acquisition of data: M.Z and P.D.
Analysis and interpretation: P.F and R.M.
Drafting of the manuscript for important intellectual content: G.B and M.H.
Statistical analysis: P.F.
Administrative, technical, and material support: S.C.
Study supervision: M.G.

Ethics

On behalf of all co-authors, I hereby confirm that I have reviewed and complied with the relevant instructions set forth in the Instructions to Authors, the Ethics in Publishing policy, and the Conflicts of Interest disclosure.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Data Availability

All data are available upon a reasonable request.

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